Evaluation of *Pangasius djambal* Bleeker 1846 and *Pangasianodon hypophthalmus* (Sauvage 1878) Hybrids: Biometric, Growth, and Ovarian Maturation

Rudhy Gustiano[1]*, Anang H. Kristanto[2], Evi Tahapari[3], and Bambang Iswanto[3]

[1]*Balai Penelitian dan Pengembangan Budidaya Air Tawar, Jl. Sempur No. 1, Bogor 16154*
*Telp. (0251) 8313200; Faks. (0251) 8327 890; *E-mail: rgustiano@yahoo.com*
[2]*Pusat Penelitian dan Pengembangan Perikanan Budidaya, Jl. Pasar Minggu No. 20, Jakarta 12540*
[3]*Balai Penelitian Pemuliaan Ikan, Jl. Raya Sukamandi, Subang 41256*

Diajukan: 5 November 2011; Diterima: 18 April 2012

ABSTRAK

Evaluasi Karakter Biometrik, Pertumbuhan, dan Perkembangan Telur Hibrida *Pangasius Djambal Bleeker* 1846 dan *Pangasianodon Hypophthalmus* (Sauvage 1878). Rudhy Gustiano, Anang H. Kristanto, Evi Tahapari, dan Bambang Iswanto. Program hibridisasi telah lama digunakan untuk meningkatkan produksi perikanan, lebih dari tiga dekade. Perhatian besar terhadap program hibridisasi merupakan kekhawatiran pengaruhnya terhadap populasi alami. Oleh karena itu, diperlukan penyediaan alat untuk mengidentifikasi secara cepat dan akurat di lapang. Kajian ini bertujuan untuk meneliti karakter biometrik, keragaan pertumbuhan, dan perkembangan telur *Pangasius djambal* dan *Pangasianodon hypophthalmus* dan hibridanya. Analisis morfologi secara lengkap dilakukan pada hibrida. Uji pertumbuhan di kolam tanah dan pengamatan terhadap perkembangan telur juga diamati. Hasil pengamatan morfometrik dan meristik memperlihatkan bahwa hibrida memiliki karakter antara kedua induk asli, kecuali jumlah gigi tapis insang yang lebih sedikit dibandingkan dengan tetuanya. Pengujian pertumbuhan di kolam tanah memperlihatkan hibrida memiliki pertumbuhan yang lebih cepat dibandingkan dengan tetuanya, sedangkan perkembangan telur hibrida menunjukkan abnormal. Perkembangan telur tidak pernah mencapai kematangan sempurna, sebagian besar rusak dan berlemak. Dari kajian yang dilakukan, dapat dikemukakan bahwa hibrida *P. djambal* and *P. hypophthalmus* adalah hibrida yang sesungguhnya. Hibrida ini dapat digunakan sebagai benih sebar untuk kegiatan produksi budi daya karena dapat dideteksi dengan mudah dari kedua tetuanya, lebih cepat tumbuh, dan tidak dapat berkembang biak.

Kata kunci: *Genetik, hibridisasi, biometrik, pertumbuhan, telur, Pangasiidae*.

ABSTRACT

Hybridization program has been used to increase fish production for more than three decades. Concern to this program is due to the problem of potential impact on wild population. Hence, it is urgently needed to provide quick identification tools in the field and as well as commercial aspect in term of growth rate. This study investigated biometric characters, growth performance, and ovarian development of *Pangasius djambal* and *Pangasianodon hypophthalmus* and their reciprocal hybrids. A detailed morphological analysis was done on the hybrids of *P. djambal* and *P. hypophthalmus*. Earthen pond on the growth comparison as well as the ovarian development was also observed. The results of morphometric and meristic showed that the reciprocal hybrids have intermediate characters except for gill rakers numbers in which lower than that of parental species. Growth analysis showed that hybrids has better performance compare to the parental one, while ovarian development of hybrids was developed abnormal. No oocytes reached vitellogenesis and showed major atresia and fatty. It can be concluded that hybrids of *P. djambal* and *P. hypophthalmus* is true hybrids. The hybrids can be used as final seed for aquaculture production due to clearly identify from parents, superior growth performance and steril.

Keywords: Genetic, hybridization, biometric, growth, ovarian, Pangasiidae.

INTRODUCTION

Efforts of breeding practices has been done to increase aquaculture production such as interspecific cross-breeding in fish which may lead hybrid with valuable characteristics for aquaculture such as sterility, monosex population, heterosis or growth. In contrast with the abundant information on the hybridisation in other cultured fish families, in particular cichlids, salmonids, cyprinids and ictalurids (Sneed, 1971; Moav, 1976; Wohlfart and Hulata, 1981; Chevassus, 1979, 1983), reports on hybridization of Pangasiid catfish are reported by Iswanto and Tahapari (2011). Catfishes of the family Pangasiidae are of great economic importance in Southeast Asia region such as *Pangasius djambal* in Indonesia (Legendre *et al.*, 2000;

Gustiano *et al*. 2003), *Pangasius bocourti* in Vietnam (Hung *et al*., 1999) and *Pangasianodon hypophthalmus* (senior of *P. Sutchi*) (Tarnchalanukit, 1986). Therefore, it was decided to evaluate the effect of hybrid vigour on the artificial hybridisation in Pangasiid catfishes.

Enzymatic system represented as protein total allowed to differentiate easily and quickly *Pangasius djambal* from *P. hypophthalmus* (Legendre *et al*., 2000). However, there is no genetic data available for the hybrids so far. It is really important to provide quick identification tools such as biometrical characters in the field as well as sterility since the possible use of hybrids in aquaculture face the problem of potential impact, genetic deterioration on wild population. This study of biometrical characters, growth performance, and ovarian development of *P. djambal* and *P. hypophthalmus* and their hybrids was to examine the potential and risks of the hybrids for final seed for aquaculture.

MATERIALS AND METHODS

Biometrics

Specimens examined in this biometric analysis including *P. djambal*: 114 specimens, 142 to 635 mm standard length (SL) collected from the main rivers in Java, Sumatra, and Kalimantan in Indonesia. *P. hypophthalmus*: 31 specimes, 147-630 mm SL originated from fish culture in Mekong River, Vietnam and fish culture in West Java in Indonesia.

Sixty three specimens of *P. djambal*, 22 of *P. hypophthalmus* deposited in the Museum Zoologicum Bogoriense (MZB), Cibinong, Indonesia and 43 specimens of *P. djambal*, 32 of *P. hypophthalmus* specimens in the Muséum National d'Histoire Naturelle (MNHN), Paris, France.

Hybrids of female *P. djambal* x male *P. hypophthalmus* : 45 specimens, 133-490 mm SL. Hybrids of male *P. hypophthalmus* x female *P. djambal*: 45 specimens, 129-473 mm SL.

All of the hybrids specimens were from artificial breeding performed at Research Institute for Freshwater Fisheries (RIFF) station in Sukamandi, Indonesia. Identification of specimens of *P. djambal* and *P. hypophthalmus* was done following Gustiano (2003).

The following anatomical abbreviations are used: SL, standard length, HL, head length. Body length was measured using a graduated ruler of one meter. Thirty two measurements were made using dial calliper following Pouyaud *et al*. (1999). Three additional measurements were done: width of pectoral spine, measured at base of second dorsal spine; anterior width of snout, taken between the borders of anterior nostril; posterior width of snout, taken between the borders of posterior nostril. Total number of gill rakers on the first branchial arch, number of dorsal, anal, pectoral and pelvic fin rays were counted. Morphological observations include the shape of the swimbladder, the shape of palatine and vomerine tooth patches.

Growth Evaluation

Pangasius djambal, *P. hypophthalmus* and their reciprocal hybrids were examined for the growth analysis. The growth comparison study were performed in the earthern pond, sized 200 m^2 at RIFF station in Sukamandi, Indonesia. Initial size of the fish was 30 2.0 g. Density used was 100 fish per pond. The fish were fed 3% of total biomass daily using artificial feed containing 28% protein. During eight month rearing period, monthly random sampling was done on 20% of population and body weight measured

Formula for the specific growth rate following Huisman (1976):

$$SGR = \frac{\frac{\ln Wt}{\ln Wo} \times 100\%}{t}$$

SGR= daily individual spesific growth (%/day)
Wt = body weigth at the end of experiment (gram)
Wo = initial body weight (gram)
t = rearing period (day)

Ovarian Development

A hundred specimens of 18 months old with weight 1.100±56.83 g were used for the study. The ovarian development was observed after dissecting examined specimens, visually for shape, colour, and the size of ovary. While microscopic observation

was done for complete oocyte and histological slides observation. Standardization of gonado somatic index (GSI) and development of oocyte stadia followed criteria developed by Siregar (1999).

RESULTS AND DISCUSSION

A principal component analysis performed on the 395 specimens using the covariance matrix for 30 measurements enable to separate *P. djambal, P. hypophthalmus*, and their hybrids. *P. djambal* was located on the positive sector of factor 2, *P. hypophthalmus* was on the negative sector of factor 2, and their hybrids were in between parental species (Figure 1).

Factor loading revealed that the second component of PCA was defined by vomerine length, palatine width, mandibular barbel length, palatine length, post-ocular length, anal fin length, maxillary barbel length, caudal peduncle depth, anal fin height (in decreasing order of importance). Further analysis showed that *P. djambal* differs from *P. hypophthalmus* by having a longer vomerine length (3.8-14% HL Vs 0.6-2.5% HL) and a larger palatine width (1.9-8.8% HL Vs 0.7-1.8% HL). While on hybrids, those two characters were intermediate, 1.2-9.4% HL for vomerine length and 1.2-4% HL for palatine width. Morphometric analysis of the specimens demonstrated clearly the presence of two species as define by Robert and Vidthayanon (1991); Vidthayanon (1993); and Gustiano (2003) as well as hybrids in between of them. This intermediate shape of hybrids suggest that the product of present hybridisation were "true" hybrids resulting from the fusions of both parents and not parthenogenesis as observed on general occasions in fish (Chevassus, 1983). Based on the morphometric data, even though the reciprocal hybrids have intermediate characters, in general their morphology were relatively hypophthalmus-like except the teeth (vomerine and palatine) that was relatively djambal-like. For meristic observation, all of *P. djambal* have 6 pelvic fin ray, while 8 on *P. hypophthalmus*. Of the hybrids, more than 97% of hypophthalmus x djambal have 7 pelvic fin ray. On the other side, the percentage was lower on the djambal x hypophthalmus where it was about 17% of the hybrids have 6 or 8 pelvic fin ray. It is also clear that the true hybrids appeared on the pelvic ray count. However, the case was contrary for gill raker number, the reciprocal hybrids have lower number than that of the parental species. The

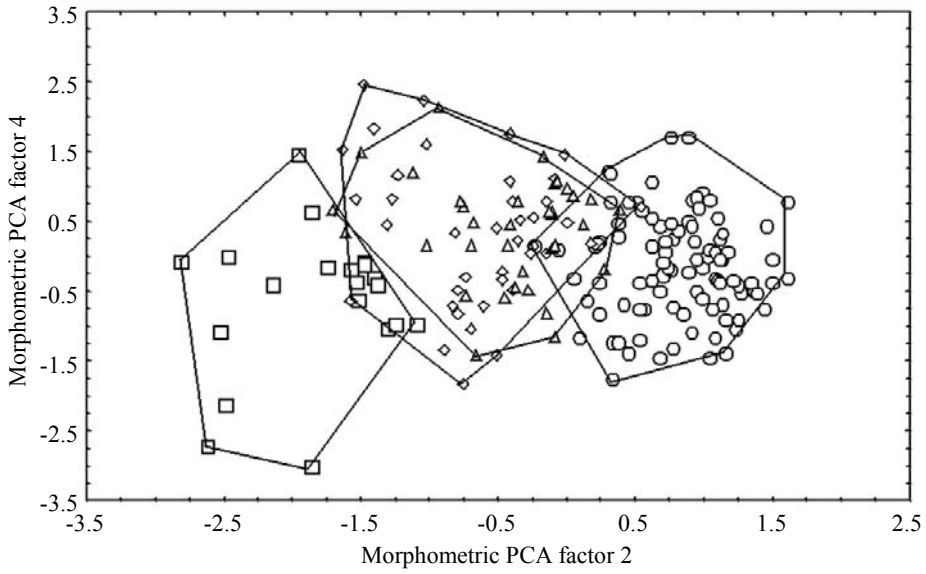

Figure 1. Plot of the second principal component (factor 2) versus the fourth principal component (factor 4) taken from a principal component analysis of 30 metric measurements on 395 specimens. ○ = *P. djambal*, □ = *P. hypophthalmus*, ◊ = *P. hypophthalmus* x *P. Djambal*, Δ = *P. djambal* x *P. hypophthalmus.*

only reason for the gill raker number was probably due to the recessive evidence.

Several characters enable to separate between the hybrids and parental species, especially for pelvic fin rays. The results are very useful in providing cheap and quick identification tool in field rather than other genetic analysis, such as enzymatic and DNA. Hence, the result from this study can also be used as a model to analysis other intergeneric hybridisation in which there is no concern too much about homozygosity.

For the growth comparison among the different groups, average body weight during eight months rearing period is given on Table 1.

At the end of experiment, the hybrids of female *P. hypophthalmus* x male *P. djambal* reached 852.3 g body weight, followed by female *P. djambal* x male *P. hypophthalmus* (764.7 g), *P. hypophthalmus* x *P. hypophthalmus* (665.5 g), *P. djambal* x *P. djambal* (524.7 g).

Body weigth is influenced by genetic, environmental (such as dissolved oxygen, temperature, feed, etc.) and their interaction (Tave, 1993). During the first six months of the study, dissolved oxygen ranged between 3-6 ppm, and temperature was 26-31^{o}C. However, thereafter water quality became worse indicated by low response of feeding, colour and odour of the water in the ponds. To avoid further negative effect, the water in the bottom part was pumped out and then substituted by fresh water. Results on spesific growth rate of the different group based on monthly sampling is presented on Table 2.

Refering to Table 2, it shows normal phenomena in the fish growth that young fish has a higher spesific growth rate compared to the old fish. Among tested groups, the hybrids performed better spesific growth rate after six month rearing period in which the water quality became poor. This result indicates that there is a correlation with the genetic and environmental interaction factors. It is clearly defined that *P. hypophthalmus* is an air breathing catfish but not for *P. djambal.* Therefore, *P. hypophthalmus* is more adaptable to the poor water quality than *P. djambal.* Hybridization has improved performance of the hybrids compared to *P. djambal* meaning there is a gene from *P. hypophthalmus* transfered to the hybrids. Evenmore, the hybrids are better than both parental species. Confirming to other study done in floating

Table 1. Average body weight (g) during eight month rearing period.

Rearing period (month)	♀ *P. hypophthalmus* x ♂ *P. hypophthalmus* (g)	♀ *P. djambal* x ♂ *P. hypophthalmus* (g)	♀ *P. djambal* x ♂ *P. djambal* (g)	♀ *P. hypophthalmus* x ♂ *P. djambal* (g)
Initial time	30.0±2.00	30.0±2.00	30.0±2.00	30.0±2.00
1	52.2±17.45	167.5±24.58	175.6±59.80	125.4±12.31
2	124.1±34.03	241.1±55.96	254.1±52.89	195.5±33.59
3	178.4±49.61	332.2±72.45	330.1±05.01	254.6±38.72
4	310.0±94.87	509.8±125.49	496.5±160.63	429.9±65.19
5	373.0±123.99	550.8±102.45	497.5±55.31	497.7±79.66
6	418.3±131.27	651.1±179.12	439.4±152.92	687.8±96.29
7	469.4±92.30	672.6±169.80	492.5±147.83	752.6±135.27
8	665.5±128.13	764.7±157.36	524.7±151.96	852.3±123.63

Table 2. Spesific growth rate (%/day) based on monthly sampling.

Month	♀ *P. hypophthalmus* x ♂ *P. hypophthalmus* (g)	♀ *P. djambal* x ♂ *P. hypophthalmus* (g)	♀ *P. djambal* x ♂ *P. djambal* (g)	♀ *P. hypophthalmus* x ♂ *P. djambal* (g)
1	3.87	5.0	5.0	4.73
2	2.36	2.68	2.71	2.58
3	1.69	1.89	1.89	1.81
4	1.40	1.55	1.52	1.48
5	1.16	1.24	1.21	1.21
6	0.98	1.05	0.99	1.06
7	0.86	0.91	0.86	0.93
8	0.79	0.81	0.76	0.82

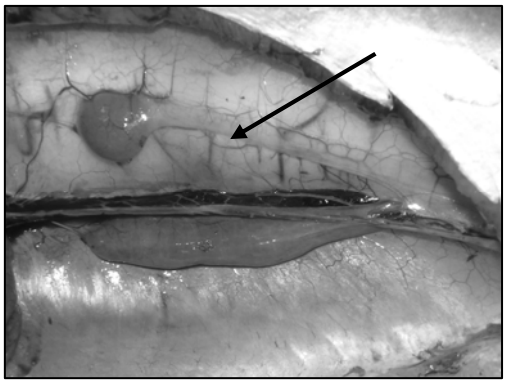

Figure 2. Abnormal ovary.

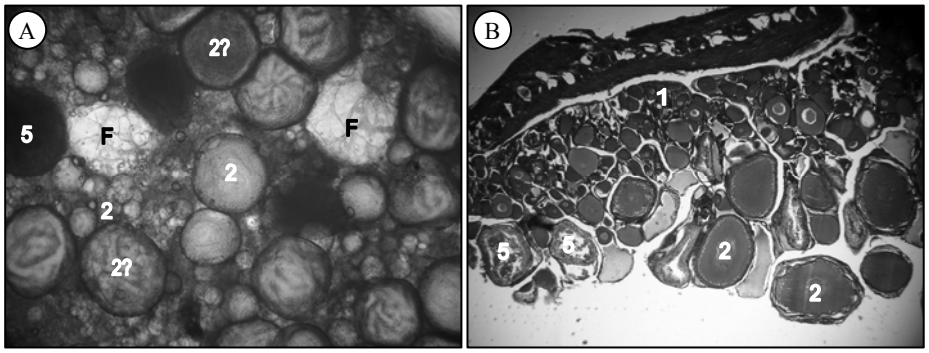

Figure 3. Intraovarian oocyte based on whole oocyte (A) and histological slide (B). 1 = 1st stadium oocyte; 2 = 2nd stadium oocyte; 2? = 2nd stadium initiating atresia; 5 = atretic oocyte (5th stadium oocyte); F = fat.

net cages using one month old fry for six weeks (Utami *et al.*, 2005), the result of the recent study shows similar result. Iswanto and Tahapari (2011) worked on similar hybridization on Pangasiids catfish reported that hybrids showed faster embryonal development than the parental strains.

Visual observation of ovarian development on hybrids showed that the sexual differentiation of ovaries were normal. Compare to the parental species, 21% of ovary hybrids performed abnormal shape (unequal) between the right and left side (Figure 2). The results also showed that the ovary were very small (0.23-0.76 mm), some of which were rudimentary, and the intraovarian oocytes developed as stadium 2. In addition, no oocytes reached vitellogenesis, and some showed major atresia (stadium 5) and fatty (Figure 3). Aside of that oocyte stadium 1 was also exist with diameter less than 0.25 mm. Based on the data and information collected, the present study ensure that there was no ecological risk appeared from hybridization since the ovarian developed abnormally.

CONCLUSION

The reciprocal hybrids have intermediate characters, except lower number of gill rakers than that of parental species. It is clear that the true hybrids have seven pelvic fin rays. Hybrids have better specific growth rate than that of parental species and steril. Since hybrids of *P. djambal* and *P. hypophthalmus* is true hybrids, it can be used as final seed for aquaculture production due to clearly identify from parents, superior growth performance and steril.

ACKNOWLEDGEMENTS

The author thanks J. Slembrouck, M. Legendre, L. Pouyaud, Sudarto for their help in collecting fish samples. This paper forms part of the INCO.DC PROJECT "Catfish Asia" financed by the European Union (contract IC 18-CT 96-0043) and RDIFA.

REFERENCES

Chevassus, B. 1979. Hybridization in salmonids results and perspective. Aquaculture 17:113-128.

Chevassus, B. 1983. Hybridization in fish. Aquaculture 33:245-262.

Gustiano, R. 2003. Taxonomy and Phylogeny of Pangasiidae catfishes from Asia. Ph.D Thesis, KU Leuven, Belgium. 298 p.

Gustiano, R., Sudarto, and L. Pouyaud. 2003. How to Recognise *Pangasius djambal*?. p. 3-14. *In* J. Slembrouck, O. Komarudin, Maskur, and L. Legendre (*eds.*) Technical Manual for Artificial Propagation of the Indonesian Catfish, *Pangasius djambal*. Karya Pratama, Jakarta.

Hung, L.T., B.M. Tam, P. Cacot, and J. Lazard. 1999. Larval rearing of the Mekong catfish *Pangasius bocourti* (Pangasiidae, Siluridae): Subtitution of Artemia nauplii with live and artificial feed. Aquat. Living Resour. 12:229-232.

Iswanto, B. and E. Tahapari. 2011. Embryogenesis and larval development of Pangasius hybrids produced from female *Pangasianodon hypophthalmus* (Sauvage, 1878) and male *Pangasius djambal* Bleeker, 1863. J. Ris. Akuakultur 6:169-186.

Legendre, M., L. Pouyaud, J. Slembrouck, R. Gustiano, A.H. Kristanto, J. Subagya, O. Komarudin, Sudarto, and Maskur. 2000. *Pangasius djambal*: A new candidate species for fish culture in Indonesia. IARD J. 22:1-14.

Moav, R. 1976. Genetic improvement in the aquaculture industry. FAO Tech. Conf. on Aquaculture. Japan. FIR: Aq/Conf/76:R.9.

Pouyaud, L., G.G. Teugels, and M. Legendre. 1999. Description of new pangasiid catfish from south-East Asia (Siluriformes). Cybium 23:247-258.

Roberts, T.R. and C. Vidthayanon. 1991. Systematic revision of the Asian catfish family Pangasiidae with biological observation and description of three new species. Proc. Acad. Nat. Sci. Philad. 143:97-144.

Siregar, M. 1999. Stimulasi pematangan gonad bakal induk betina ikan Jambal Siam (*Pangasius hypoph-thalmus*), dengan hormon HCG. Tesis program pasca sarjana IPB. Bogor. 120 hlm.

Sneed, K.E. 1971. Some current North American work in hybridization and selection of cultured fish. Report FAO/UNDP (TA) 2926:143-150.

Tarnchalanukit, W. 1986. Experimental hybridization between catfishes of families Clariidae and Pangasiidae in Thailand. Environ. Biol. Fish. 16:317-320.

Tave, D. 1993. Genetics for Fish Hatchery managers. AVI Publ. Comp. Inc, NY, USA 2nd ed. 418 p.

Utami, R., B. Gunadi, D. Ariyanto, and Sularto. 2005. Performance of fingerling of *Pangasius hypophthalmus, Pangasius djambal* and their hybrids reared in net cages. Abstract presented at World Aquaculture Society 2005, Bali, Indonesia.

Wohlfart, G.W. and G.I. Hulata. 1981. Applied genetics of Tilapias. ICLARM Studies and Review 6. Manila: ICLARM.

Vidthayanon, C. 1993. Taxonomic revision of catfish family Pangasiidae. Ph.D. Thesis. Tokyo University of Fisheries. 203 p.